



## Novel hexarelin analogs stimulate feeding in the rat through a mechanism not involving growth hormone release

Antonio Torsello <sup>a</sup>, Marina Luoni <sup>a</sup>, Francesca Schweiger <sup>a</sup>, Roberta Grilli <sup>a</sup>,  
Margherita Guidi <sup>a</sup>, Elena Bresciani <sup>a</sup>, Romano Deghenghi <sup>b</sup>, Eugenio E. Müller <sup>a</sup>,  
Vittorio Locatelli <sup>a,\*</sup>

<sup>a</sup> Department of Pharmacology, University of Milan, via Vanvitelli 32, 20129 Milan, Italy

<sup>b</sup> Europeptides, Argenteuil, France

Received 18 June 1998; revised 10 September 1998; accepted 15 September 1998

### Abstract

Growth hormone-releasing peptides (GHRPs) are a class of small peptides that stimulate growth hormone (GH) release in several animal species, including the human. Moreover, GHRPs injected into the brain ventricles stimulate feeding in the rat. The aim of this study was to evaluate the GH-releasing properties of a series of novel GHRP analogs and the possible existence of functional correlations between the GH-releasing activity and the effects on feeding behavior. Two well-known hexapeptides, GHRP-6 and hexarelin, given s.c., dose dependently stimulated both GH release and feeding behavior in satiated rats. However, in a series of tri-, penta- and hexapeptide analogs of hexarelin, some compounds were active either on GH release or on eating behavior. Interestingly, even minor structural modifications resulted in major changes of the pharmacological profile. We conclude that GHRPs have orexigenic properties after systemic administration which are largely independent from the effects they exert on GH release. © 1998 Elsevier Science B.V. All rights reserved.

**Keywords:** Hexarelin; GHRP (growth hormone-releasing peptide); GH (growth hormone)-secretagogue; Feeding; GH (growth hormone) secretion

### 1. Introduction

The growth hormone-releasing peptides (GHRPs) are synthetic compounds that reportedly stimulate GH secretion in several animal species as well as in humans (Bowers et al., 1984, 1990; Croom et al., 1984; Kraft et al., 1984; Doscher et al., 1984; Ilson et al., 1989; Malozowski et al., 1991). They are active after i.v., s.c., intranasal, or oral administration (Walker et al., 1990; Bowers et al., 1992; Bowers, 1993; Hayashi et al., 1993). GHRPs act directly on the pituitary, but there is also evidence that their endocrine action may be exerted on the hypothalamus (Bowers et al., 1991; Dickson et al., 1993). Consistent with this, a specific receptor for GHRPs has been detected in the hypothalamus (Pong et al., 1996; Howard et al., 1996) and systemic administration of GHRPs induces the expression of c-Fos protein in the arcuate nucleus of the hypothalamus (Dickson et al., 1993).

In addition to triggering GH release, GHRP-6 and KP-102 (*D*-Ala-*D*-βNal-*Ala*-Trp-*D*-Phe-Lys-NH<sub>2</sub>) injected into the brain ventricles stimulate food intake in the satiated rat (Locke et al., 1995; Okada et al., 1996), an effect reminiscent of that of i.c.v.-injected GH-releasing hormone (GHRH) (Vaccarino et al., 1985). Hexarelin, another GHRP-6 analog and strong GH releaser (Deghenghi et al., 1994) was also shown to stimulate c-Fos expression in the arcuate nucleus, and this occurred after systemic administration of the peptide (Dickson et al., 1995). Based on this observation, we reasoned that hexarelin could have been able to stimulate feeding behavior in rats after systemic administration also.

We recently characterized the GH-releasing activity of a family of hexarelin peptidic analogs, some of which proved to be either similar to or even more potent and effective than the parent compound (Deghenghi et al., 1995). Hence, we thought it would be of interest to investigate the effects of these compounds on food intake and whether or not these effects depend on and/or correlate with the GH-releasing activity. To this end, we now examined the effects of systemic administration of GHRP-6, hexarelin, and its

\* Corresponding author. Tel.: +39-02701-46215; Fax: +39-02701-06838; E-mail: vittorio.locatelli@imiucca.csi.unimi.it

novel analogs on plasma GH levels in neonatal male rats and compared such effects with those on food intake evaluated in free-feeding young-adult male rats.

## 2. Materials and methods

### 2.1. Animals

Sprague-Dawley rats (Charles River, Calco, Italy) were housed in our facilities (see also below) under controlled conditions ( $22 \pm 2^\circ\text{C}$ , 65% humidity, artificial light from 0600–2000 h). A standard dry diet and water were available ad libitum. All the experiments were performed in accordance with the Italian Guidelines for the Use of Animals in Medical Research.

### 2.2. Peptides

All peptides reported upon in this paper were prepared by conventional solid phase synthesis by one of us (R.D.). Each peptide was purified to a purity of at least 98% by high-performance liquid chromatography.

### 2.3. Experimental procedure

#### 2.3.1. Feeding behavior

In these experiments, young-adult male rats (weighing 200–250 g) were used. After 1 week of acclimation in individual cages, and daily training to mimic the experimental procedure, sated rats were treated in the morning (1000–1100 h) with s.c. graded doses (20–640  $\mu\text{g}/\text{kg}$ ) of GHRP-6 or hexarelin, or one of the test compounds (320  $\mu\text{g}/\text{kg}$ ), or isovolumetric amounts of physiological saline. Immediately after the injection, the animals were returned to their home cages, which contained a known amount of rat chow. Every hour and for the following 6 h, the remaining food was carefully collected and weighed to the nearest 0.1 g. Food intake was normalized for the body weight of the rats.

#### 2.3.2. GH-releasing activity

We have previously shown that the 10-day-old rat is an animal model well-suited for the evaluation of the GH-releasing activity of GH secretagogues (Cella et al., 1985; Cozzi et al., 1986; Cella et al., 1988). In particular, we have determined that in this animal model, GH plasma levels peak 15 min following the s.c. administration of hexarelin or GHRP-6 (Deghenghi et al., 1994). Based on these premises, we chose the infant rat for evaluating the effects on plasma GH secretion of a number of GHRP-6 and hexarelin analogs.

Male rat pups with their dams were received in our facilities on postnatal day 7. The pups were immediately separated from their mothers and randomly redistributed to the dams, so that each one nurtured eight pups. On postna-

tal day 10, the pups were separated from their mothers. After 1 h they were given a fixed dose (320  $\mu\text{g}/\text{kg}$ ) of one of the test compounds or isovolumetric amounts of physiological saline s.c. and were killed 15 min later by decapitation. Trunk blood was collected, immediately centrifuged and plasma samples were stored at  $-20^\circ\text{C}$  until assayed for the determination of GH concentrations.

### 2.4. GH assay

Plasma GH concentrations were measured by radioimmunoassay using materials kindly provided by the NHPP, NIDDK, NICHD, USDA, USA. Values are expressed in terms of rat-GH-RP-2 standard (potency 2 IU/mg) as ng/ml of plasma. The minimum detectable value of rat GH was 1.0 ng/ml; intra-assay variability was 6%. To avoid inter-assay variations, all samples from each experiment were run in one single assay.

### 2.5. Statistical analysis

The statistical significance of differences between GH plasma levels in control and experimental groups was evaluated with Dunnett's *t*-test for multiple comparisons, preceded by an analysis of variance (ANOVA). Statistical analysis of food intake values was performed by One-way ANOVA followed by the Bonferroni test for multiple

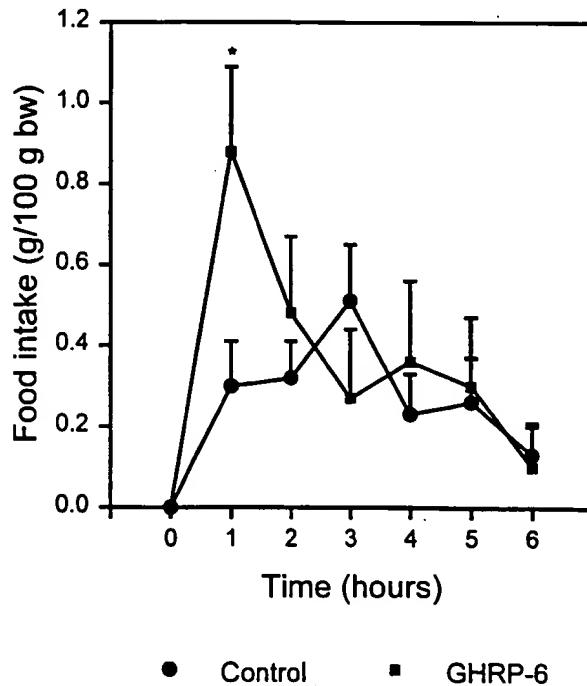


Fig. 1. Time course of GHRP-6 stimulation of feeding behavior. Young-adult male rats (eight rats per group) were injected s.c. with 320  $\mu\text{g}/\text{kg}$  GHRP-6 or isovolumetric amounts of physiological saline and food intake was evaluated every hour for the 6 h following treatment. Food intake was normalized for the body weight of the rats. Data are expressed as the means  $\pm$  S.E. of 24 determinations from three independent experiments. \*  $P < 0.05$  vs. saline.

comparisons. A *P*-value of less than 0.05 was considered significant.

### 3. Results

#### 3.1. Effects on feeding behavior

##### 3.1.1. GHRP-6 and hexarelin

In young-adult male rats, s.c. administration of GHRP-6 (320 µg/kg) caused a significant increase in the amount of food eaten in the first hour (Fig. 1). In the following 5 h, food intake was similar in GHRP-6 and saline-treated rats. Administration of increasing doses of GHRP-6 (20–640 µg/kg, s.c.) revealed that the 80-µg/kg dose was effective and that stimulation was maximal with the 160-µg/kg dose (Fig. 2). Again, with all doses, stimulation of feeding behavior was confined to the first post-treatment hour and then disappeared rapidly. The cumulated amount of food eaten in the 6-h period was significantly higher in the experimental groups given the 80 to 640 µg/kg GHRP-6 doses than in the controls (Fig. 2). A similar pattern was present with hexarelin, which also stimulated food intake mainly in the first post-treatment h (Fig. 3). Hexarelin effectively stimulated feeding in the first hour at

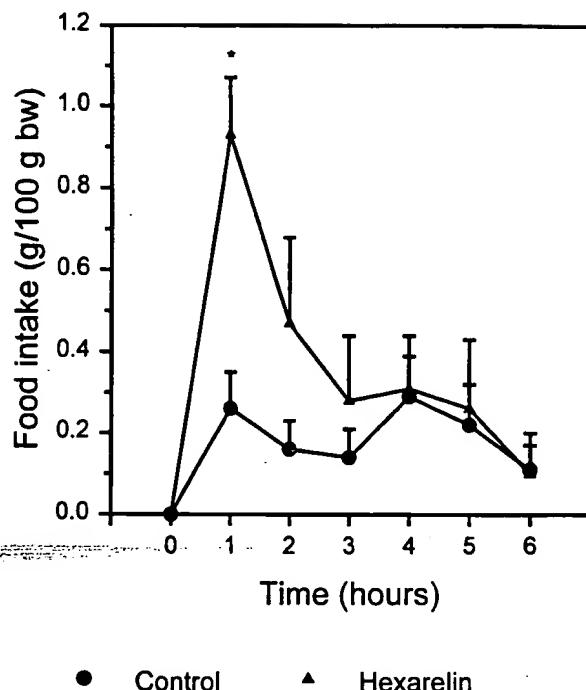


Fig. 3. Time course of hexarelin stimulation of feeding behavior. Rats were treated as described in the legend to Fig. 1. Data are expressed as means  $\pm$  S.E. of 24 determinations from three independent experiments. \*  $P < 0.05$  vs. saline.

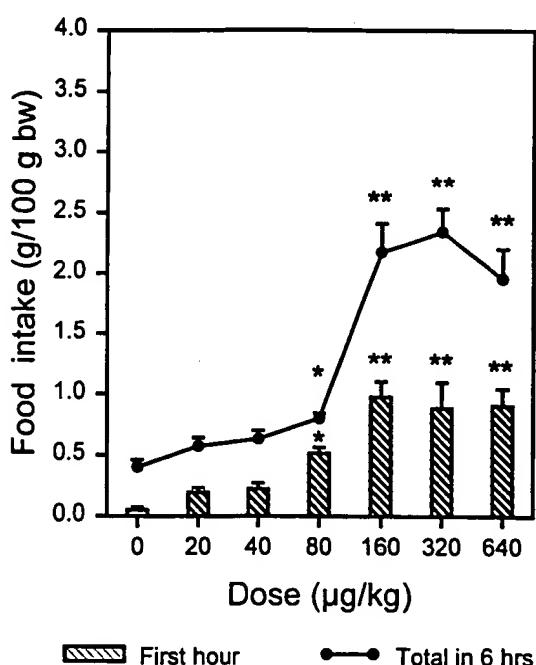


Fig. 2. Dose-response study of GHRP-6 stimulation of food intake. Young-adult male rats (eight rats per group) were injected s.c. with increasing doses of GHRP-6 or physiological saline and food intake was evaluated every hour for the 6 post-treatment h. Food intake was normalized for the body weight of the rats. Data are expressed as means  $\pm$  S.E. of 24 determinations from three independent experiments. Bars indicate the food intake in the first hour and the line is the total amount of food eaten in the 6-h observation period. \*  $P < 0.05$  and \*\*  $P < 0.01$  vs. saline.

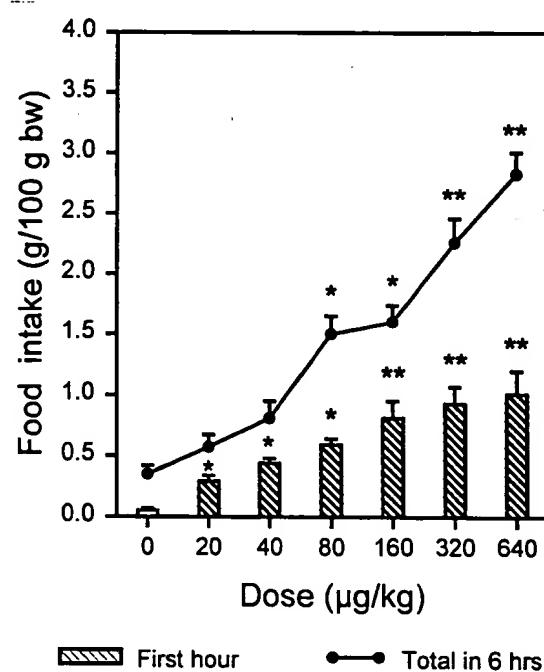


Fig. 4. Dose-response study of hexarelin stimulation of food intake. Rats were treated as described in the legend to Fig. 2. Data are expressed as means  $\pm$  S.E. of 24 determinations from three independent experiments. Bars indicate the food intake in the first hour and the line is the total amount of food eaten in the 6-h observation period. \*  $P < 0.05$  and \*\*  $P < 0.01$  vs. saline.

Table 1

Effects of hexapeptidic GHRP analogs on GH release and feeding behavior of the rat

Analog	Structure	Food intake	Plasma growth hormone (ng/ml)
Saline			15.7 ± 6.7
GHRP-6	His-D-Trp-Ala-Trp-D-Phe-Lys-NH <sub>2</sub>	Active	94.7 ± 6.8 <sup>a</sup>
Hexarelin	His-D-Trp(2-Me)-Ala-Trp-D-Phe-Lys-NH <sub>2</sub>	Active	101.9 ± 8.6 <sup>a</sup>
EP 40904	Thr-D-Trp(2-Me)-Ala-Trp-D-Phe-Lys-NH <sub>2</sub>	Active	20.1 ± 3.4
EP 40737	D-Thr-D-Trp(2-Me)-Ala-Trp-D-Phe-Lys-NH <sub>2</sub>	Inactive	346.0 ± 73.0 <sup>a</sup>

The effects on food intake were determined in young-adult male rats given a single injection of 320 µg/kg, s.c. of the test compound.

Active indicates a significant increase in the amount of food eaten in the first post-treatment hour as compared to that consumed by rats given saline. The effects on GH release were evaluated in 10-day-old male rats given 320 µg/kg, s.c., of the test compound and killed 15 min later.

Plasma GH levels are expressed as the means ± S.E. of 24 determinations from three independent experiments.

<sup>a</sup>P < 0.01 vs. saline.

### 3.1.2. GHRP analogs

In these experiments, several GHRP analogs obtained by modification or downsizing of the hexarelin structure were evaluated in terms of their ability to stimulate food intake following systemic administration. Table 1 shows that the replacement of His<sup>1</sup> in the hexarelin sequence by Thr<sup>1</sup> (EP 40904) did not modify the orexigenic effect of the compound, whereas the replacement with its isomer, D-Thr<sup>1</sup> (EP 40737), caused complete loss of this property. The second group of compounds tested consisted of six pentapeptides. In the first, EP 51215, the C-terminal tetrapeptide sequence of hexarelin was replaced by the tripep-

tide GAB-D-Trp(2-Me)-D-Trp(2-Me). EP 51215 efficiently stimulated food intake but its analog EP 50477, in which D-Trp(2-Me)<sup>3</sup> had been replaced by D-Trp<sup>3</sup>, was inactive. A similar loss of activity was observed when the GAB<sup>1</sup> group of EP 50885 was replaced by tranexamyl<sup>1</sup> in the EP 50887 and when the Trp(2-Me)<sup>4</sup> in EP 51216 was replaced by its isomer D-Trp(2-Me)<sup>4</sup> in EP 60761 (Table 2).

Other five pentapeptides were obtained by modifications of the sequence of EP 50885 (Table 3). The N-terminal dipeptide, Phe-Lys, was retained in all of them, and activity was preserved when the GAB<sup>1</sup> was replaced by

Table 2  
Effects of pentapeptidic analogs of hexarelin on GH release and feeding behavior of the rat

Analog	Structure	Food intake	Plasma growth hormone (ng/ml)
Saline			21.1 ± 7.6
EP 51215	GAB-D-Trp(2-Me)-D-Trp(2-Me)-Phe-Lys-NH <sub>2</sub>	Active	155.4 ± 19.7 <sup>a</sup>
EP 50477	GAB-D-Trp(2-Me)-D-Trp-Phe-Lys-NH <sub>2</sub>	Inactive	110.1 ± 10.0 <sup>a</sup>
EP 50887	Tranexamyl-D-Trp(2-Me)-D-βNal-Phe-Lys-NH <sub>2</sub>	Inactive	24.9 ± 4.7
EP 50885	GAB-D-Trp(2-Me)-D-βNal-Phe-Lys-NH <sub>2</sub>	Active	145.7 ± 9.0 <sup>a</sup>
EP 51216	GAB-D-Trp(2-Me)-D-Trp(2-Me)-Trp(2-Me)-Lys-NH <sub>2</sub>	Active	165.4 ± 18.5 <sup>a</sup>
EP 60761	GAB-D-Trp(2-Me)-D-Trp(2-Me)-D-Trp(2-Me)-Lys-NH <sub>2</sub>	Inactive	91.2 ± 9.0 <sup>a</sup>

Determinations done as described in the legend to Table 1.

Abbreviations: GAB = γ-aminobutyryl; βNal = β-(2-naphthyl)alanine.

<sup>a</sup>P < 0.01 vs. saline.

Table 3

Effects of analogs preserving the N-terminal dipeptide, Phe-Lys, on GH release and feeding behavior of the rat

Analog	Structure	Food intake	Plasma growth hormone (ng/ml)
Saline			17.7 ± 3.7
EP 41612	INIP-D-βNal-D-Trp-Phe-Lys-NH <sub>2</sub>	Active	147.4 ± 13.5 <sup>b</sup>
EP 41613	INIP-D-βNal-D-βNal-Phe-Lys-NH <sub>2</sub>	Active	139.4 ± 8.3 <sup>b</sup>
EP 41614	INIP-D-Trp(2-Me)-D-Trp-Phe-Lys-NH <sub>2</sub>	Active	156.3 ± 13.0 <sup>b</sup>
EP 41615	INIP-D-Trp(2-Me)-D-βNal-Phe-Lys-NH <sub>2</sub>	Active	151.9 ± 16.1 <sup>b</sup>
EP 41616	Imidazolylacetyl-D-Trp(2-Me)-D-Trp-Phe-Lys-NH <sub>2</sub>	Inactive	60.3 ± 8.1 <sup>a</sup>

Determinations done as described in the legend to Table 1.

Abbreviations: INIP = isonipepticinyl; βNal = β-(2-naphthyl)alanine.

<sup>a</sup>P < 0.05 vs. saline.

<sup>b</sup>P < 0.01 vs. saline.

**Table 4**  
Effects of tripeptides on GH release and feeding behavior of the rat

Analog	Structure	Food intake	Plasma growth hormone (ng/ml)
Saline			19.2 ± 4.7
EP 51390	AIB-D-Trp(2-Me)-Trp(2-Me)-NH <sub>2</sub>	Inactive	20.1 ± 7.7
EP 51389	AIB-D-Trp(2-Me)-D-Trp(2-Me)-NH <sub>2</sub>	Active	174.2 ± 25.9 <sup>b</sup>
EP 60022	GAB-D-Trp(2-Me)-D-Trp(2-Me)-NH <sub>2</sub>	Inactive	15.0 ± 1.5
EP 60274	GAB-D-Trp(2-Me)-Trp(2-Me)-NH <sub>2</sub>	Inactive	36.0 ± 9.5 <sup>a</sup>

Determinations done as described in the legend to Table 1.

Abbreviations: GAB = γ-aminobutyryl; AIB = aminoisobutyryl.

<sup>a</sup>P < 0.05 vs. saline.

<sup>b</sup>P < 0.01 vs. saline.

the INIP<sup>1</sup>, but not the imidazolylacetyl<sup>1</sup> group. The four analogs containing the GAB<sup>1</sup> (i.e., EP 41612, EP 41613, EP 41614, and EP 41615) effectively stimulated food intake whereas EP 41616 was inactive (Table 3). D-βNal<sup>2</sup> could successfully replace D-Trp(2-Me)<sup>2</sup>; and similarly, D-Trp<sup>3</sup> was interchangeable with D-βNal<sup>3</sup> without affecting the properties of the molecule (Table 3).

Among the tripeptides, only EP 51389 significantly stimulated food intake, whereas its analogs with Trp(2-Me)<sup>3</sup> (EP 51390) substituted for the D-Trp(2-Me)<sup>3</sup> group or with GAB<sup>1</sup> (EP 60022) substituted for AIB<sup>1</sup>, or both substitutions (EP 60274), were inactive (Table 4).

Neither GHRP-6 nor hexarelin or the other peptide analogs caused any gross behavioral change.

### 3.2. Effects on GH release

#### 3.2.1. GHRP analogs

The effects of the two hexapeptide analogs, EP 40737 and EP 40904, on GH release were the exact opposite of the effects on feeding behavior. EP 40737 proved to be a very effective GH secretagogue whereas EP 40904 was inactive (Table 1). All the pentapeptides in Tables 2 and 3 efficiently stimulated GH release, the sole exception being EP 50887, the analog of EP 50885 in which the GAB<sup>1</sup> group had been replaced by tranexamyl<sup>1</sup>, which had no activity (Table 2). Among the tripeptides tested, only EP 51389 effectively stimulated GH release, whereas the other analogues were weakly active (EP 60274) or ineffective (EP 51390 and EP 60022) (Table 4).

## 4. Discussion

We have demonstrated that s.c. administration of GHRP-6 or hexarelin causes a dose-dependent stimulation of feeding in sated rats. Similarly, a number of GHRP analogs that we have tested had feeding-stimulating properties after systemic administration. With all these compounds, the effect was short-lived, being evident only within 1 h after treatment. Our data support and extend previous findings showing that GHRP-6 stimulates feeding

behavior in the rat after i.c.v. administration (Locke et al., 1995). It was proposed by these authors that GHRP-6 stimulation of feeding could be largely or entirely independent of its effects on plasma GH levels. In the present study, several GHRP analogs consistently stimulated both GH secretion and food intake, others were effective on plasma GH levels only and, conversely, one of them (EP 40904) selectively stimulated feeding behavior without causing GH release. Collectively, these data provide the first unequivocal demonstration that the ability of GHRPs to stimulate eating behavior is not paralleled by the ability to trigger GH release.

Data from the literature support the view that GHRP-6 stimulates eating behavior by a direct action on the hypothalamus. In fact, a specific receptor for GHRPs is expressed in the hypothalamus (Pong et al., 1996; Howard et al., 1996), and, in the rat, systemic administration of GHRPs induces the expression of Fos protein in putative GHRH neurons of the arcuate nucleus (Dickson et al., 1993). The activation of these GHRH neurons could be involved in the orexigenic properties of GHRPs, since strong evidence indicates that GHRH stimulates eating behavior via a direct hypothalamic action (Vaccarino et al., 1985). However, this view is countered by the demonstration that, in the rat, the maximally effective i.c.v. doses of GHRH and KP-102 were additive, and that a GHRH antagonist while fully effective to block GHRH action did not do so for KP-102 (Okada et al., 1996). These findings suggest that different mechanisms underlie the stimulation of eating behavior by GHRPs and GHRH. In line with this view, our study showed that even minimal structural modifications of GHRP analogs affect the behavioral but not the GH-releasing properties. This was the case for EP 51216 and its analog, EP 60761, and for EP 51215 and its analog, EP 50477 (Table 2). Most dramatic was the case of EP 40904 and EP 40737, two hexarelin analogs bearing the substitution with L-Thr<sup>1</sup> and D-Thr<sup>1</sup>, respectively, for His<sup>1</sup> in the parent compound which act only on eating behavior, or on GH release, respectively. Based on the foregoing, it is possible to postulate that at least two different GHRP receptor subtypes are expressed in the rat hypothalamus, one being prevalently located on neural

structures involved in the neuroendocrine control of GH secretion (e.g., the arcuate nucleus) and the other located on those structures more involved in the regulation of feeding behavior (paraventricular nucleus, ventromedial nucleus, limbic system). This postulate is consistent with the ability of GHRPs to activate neuropeptide Y-containing neurons (Dickson and Luckman, 1997) and with the expression of GHRP-receptors also in brain locations outside the areas mainly responsible for the control of GH release (Guan et al., 1997).

GHRP-6 and hexarelin fulfil most of the requirements for potential clinical use, being reasonably selective, very effective parenterally and with a negligible toxicity profile for the therapeutic range of doses (Imbimbo et al., 1994). However, their oral availability is low (about 0.3%) (Ghigo et al., 1994), which has prompted a search for peptidyl and non-peptidyl GHRP analogs with greater oral bioavailability. Because tri- and pentapeptides usually have better oral absorption than do hexapeptides, a number of compounds obtained by downsizing and modifying the hexarelin structure have been tested on the same physiological parameters. It had been reported that practically any attempted substitution in the C-terminal tetrapeptide sequence (Ala-Trp-D-Phe-Lys-NH<sub>2</sub>) of GHRP-6 was deleterious to its GH-releasing activity (Momany et al., 1981). Interestingly, a cyclic analog of hexarelin was also totally inactive, indicating the importance of the linear structure and/or of the presence of NH<sub>2</sub> terminal functions (Deghenghi et al., 1994; Deghenghi, 1996). Based on these observations, it was tempting to consider the C-terminal tetrapeptide of GHRP-6 as a 'pharmacophore' responsible at least in part for the activity of GHRPs. In particular, the Trp and D-Phe moieties appeared to be the most sensitive to changes. However, the first exception to this proposition was provided in our study by the demonstration that the pentapeptide, EP 51216, in which only the N-terminal Lys moiety is preserved (Table 2), was fully active to stimulate both GH release and eating behavior. Further, downsizing attempts allowed the characterization of EP 51389 (Table 4), a tripeptide that retained full activity on both feeding behavior and GH release. It is intriguing that although the sequence of EP 51389 does not contain any of the amino acid residues of its parent compound, GHRP-6, the simple substitution of the N-terminal D-Trp(2-Me) with its L-isomer (EP 51390) caused the complete loss of both activities.

In conclusion, this study has shown first, that GHRPs have orexigenic properties after systemic administration in the rat. Second, GHRP stimulation of eating behavior is largely or entirely independent of the effects that these compounds exert on GH release, and the two effects are possibly based on activation of different subtypes of GHRP-receptor. Third, although the compounds are structurally related, even minor modifications of the GHRP sequence may result in striking and unpredictable variations of the pharmacological profile.

## Acknowledgements

This work was supported in part by a research grant from the Italian Ministero dell'Università e della Ricerca Scientifica e Tecnologica.

## References

- Bowers, C.Y., 1993. GH releasing peptides. Structure and kinetics. *J. Pediatr. Endocrinol.* 6, 21–31.
- Bowers, C.Y., Momany, F.A., Reynolds, G.A., Hong, A., 1984. On the in vitro and in vivo activity of a new synthetic hexapeptide that acts on the pituitary to specifically release growth hormone. *Endocrinology* 114, 1537–1545.
- Bowers, C.Y., Reynolds, G.A., Durham, D., Barrera, C.M., Pezzoli, S.S., Thorner, M.O., 1990. Growth hormone (GH)-releasing peptide stimulates GH release in normal men and acts synergistically with GH-releasing hormone. *J. Clin. Endocrinol. Metab.* 70, 975–982.
- Bowers, C.Y., Sartor, A.O., Reynolds, G.A., Badger, T.M., 1991. On the action of the growth hormone-releasing hexapeptide, GHRP. *Endocrinology* 128, 2027–2035.
- Bowers, C.Y., Alster, D.K., Frentz, J.M., 1992. The growth hormone-releasing activity of a synthetic hexapeptide in normal men and short statured children after oral administration. *J. Clin. Endocrinol. Metab.* 74, 292–298.
- Cella, S.G., Locatelli, V., De Gennaro, V., Puggioni, R., Pintor, C., Müller, E.E., 1985. Human pancreatic growth hormone (GH)-releasing hormone stimulates GH synthesis and release in infant rats, an in vivo study. *Endocrinology* 116, 574–577.
- Cella, S.G., Locatelli, V., De Gennaro, V., Bondiolotti, G.P., Pintor, C., Loche, S., Provezza, M., Müller, E.E., 1988. Epinephrine mediates the growth hormone-releasing effect of galanin in infant rats. *Endocrinology* 122, 855–859.
- Cozzi, M.G., Zanini, A., Locatelli, V., Cella, S.G., Müller, E.E., 1986. Growth hormone releasing hormone and clonidine stimulate biosynthesis of growth hormone in neonatal pituitaries. *Biochem. Biophys. Res. Commun.* 138, 1223–1230.
- Croom, W.J., Leonard, E.S., Baker, P.K., Kraft, L.A., Ricks, C.A., 1984. The effects of synthetic growth hormone releasing hexapeptide BI 679 on serum growth hormone levels and production in lactating dairy cattle. *J. Anim. Sci.* 67, 109.
- Deghenghi, R., 1996. Growth hormone releasing peptides. In: Bercu, B.B., Walker, R.F. (Eds.), *Growth Hormone Secretagogues*. Springer, New York, pp. 85–102.
- Deghenghi, R., Cananzi, M.M., Torsello, A., Battisti, C., Müller, E.E., Locatelli, V., 1994. GH-releasing activity of hexarelin, a new growth hormone releasing peptide, in infant and adult rats. *Life Sci.* 54, 1321–1328.
- Deghenghi, R., Boutignon, F., Luoni, M., Grilli, R., Guidi, M., Locatelli, V., 1995. Small peptides as potent releasers of growth hormone. *J. Pediatr. Endocrinol. Metab.* 8, 311–313.
- Dickson, S.L., Luckman, S.M., 1997. Induction of c-fos messenger ribonucleic acid in neuropeptide Y and growth hormone (GH)-releasing factor neurons in the rat arcuate nucleus following systemic injection of the GH secretagogue, GH-releasing peptide-6. *Endocrinology* 138, 771–777.
- Dickson, S.L., Leng, G., Robinson, I.C.A.F., 1993. Systemic administration of growth hormone-releasing peptide activates hypothalamic arcuate neurons. *Neurosci. Lett.* 53, 303–307.
- Dickson, S.L., Leng, G., Dyball, R.E.J., Smith, R.G., 1995. Central action of peptide and non-peptide growth hormone secretagogues in the rat. *Neuroendocrinology* 61, 36–43.
- Doscher, M.E., Baker, P.K., Kraft, L.A., Ricks, C.A., 1984. Effect of a synthetic growth hormone releasing hexapeptide (BI 679) and growth

- hormone releasing factor (GRF) on serum growth hormone levels in barrows. *J. Anim. Sci.* 59, 218.
- Ghigo, E., Arvat, E., Gianotti, L., Imbimbo, B.P., Lenaerts, V., Deghenghi, R., Carnanni, F., 1994. Growth hormone-releasing activity of hexarelin, a new synthetic hexapeptide, after intravenous, subcutaneous, intranasal, and oral administration in man. *J. Clin. Endocrinol. Metab.* 78, 693–698.
- Guan, X.M., Yu, H., Palyha, O.C., McKee, K.K., Feighner, S.D., Sirinathsinghji, D.J., Smith, R.G., Van der Ploegh, L.H., Howard, A.D., 1997. Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. *Brain Res. Mol. Brain Res.* 48, 23–29.
- Hayashi, S., Kaji, H., Abe, H., Chihara, K., 1993. Effect of intravenous administration of growth hormone-releasing peptide on plasma growth hormone in patients with short stature. *Clin. Pediatr. Endocrinol.* 2 (Suppl. 2), 69–74.
- Howard, A.D., Feighner, S.D., Cully, D.F., Arena, J.P., Liberator, P.A., Rosenblum, C.I., Hamelin, M.J., Hreniuk, D.L., Palyha, O.C., Anderson, J., Paress, P.S., Diaz, C., Chou, M., Liu, K., McKee, K.K., Pong, S.S., Chaung, L.Y., Elbrecht, A., Dashkevich, M., Heavens, R., Rigby, M., Sirinathsinghji, D.J.S., Dean, D.C., Melillo, D.G., Patchett, A.A., Nargund, R., Griffin, P.R., DeMartino, J.A., Gupta, S.K., Schaeffer, J.M., Smith, R.G., Van der Ploeg, L.H.T., 1996. Cloning of a G-protein coupled receptor for the growth hormone releasing peptide (GHRP-6) and non-peptidyl growth hormone secretagogues. *Science* 273, 974–977.
- Ilson, B.E., Jorkasky, D.K., Curnow, R.T., Stote, R.M., 1989. Effect of a new synthetic hexapeptide to selectively stimulate growth hormone release in healthy human subjects. *J. Clin. Endocrinol. Metab.* 69, 212–214.
- Imbimbo, B.P., Mant, T., Edwards, M., Amin, D., Dalton, N., Boutignon, F., Lenaerts, V., Wuthrich, P., Deghenghi, R., 1994. Growth hormone-releasing activity of hexarelin in humans. *Eur. J. Clin. Pharmacol.* 46, 421–425.
- Kraft, L.A., Baker, P.K., Doscher, M.E., Ricks, C.A., 1984. Effect of a synthetic growth hormone releasing hexapeptide on serum growth hormone levels in steers. *J. Anim. Sci.* 59, 218.
- Locke, W., Kirgis, H.D., Bowers, C.Y., Abdoh, A.A., 1995. Intracerebroventricular growth hormone-releasing peptide-6 stimulates eating without affecting plasma growth hormone responses in rats. *Life Sci.* 56, 1347–1352.
- Malozowski, S., Hao, E.N., Ren, S.G., Marin, G., Liu, L., Southers, J.L., Merriam, G.R., 1991. Growth hormone (GH) responses to the hexapeptide GH-releasing peptide and GH-releasing hormone (GHRH) in the cynomolgus macaque: evidence for non-GHRH-mediated responses. *J. Clin. Endocrinol. Metab.* 73, 314–317.
- Momany, F.A., Bowers, C.Y., Reynolds, G.A., Chang, D., Hong, A., Newlander, K., 1981. Design, synthesis and biological activity of peptides which release growth hormone in vitro. *Endocrinology* 108, 31–39.
- Okada, K., Ishii, S., Minami, S., Sugihara, H., Shibasaki, T., Wakabayashi, I., 1996. Intracerebroventricular administration of the growth hormone-releasing peptide KP-102 increases food intake in free-feeding rats. *Endocrinology* 137, 5155–5158.
- Pong, S.S., Chaung, L.Y.P., Dean, D., Nargund, R.P., Patchett, A.A., Smith, R.G., 1996. Identification of a new G-protein-linked receptor for growth hormone secretagogues. *Mol. Endocrinol.* 10, 57–61.
- Vaccarino, F.J., Bloom, F.E., Rivier, J., Vale, W., Koob, G.F., 1985. Stimulation of food intake in rats by centrally administered hypothalamic growth hormone-releasing factor. *Nature* 314, 167–168.
- Walker, R.F., Codd, E.E., Barone, F.C., Nelson, A.H., Goodwin, T., Campbell, S.A., 1990. Oral activity of the growth hormone releasing peptide His-D-Trp-Ala-Trp-D-Phe-Lys-NH<sub>2</sub> in rat, dogs, monkeys. *Life Sci.* 47, 29–36.